

## Analytical evaluation of the iron transfer from cigarette tobacco to human body

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**Abstract** It was analytically examined how great amount of total iron can be absorbed during cigarette smoking. For this purpose, tobacco taken from cigarettes of selected brands (Marlboro, L&M, and Popular) was carefully analyzed before and after smoking. In addition, the cigarette filters were checked in terms of iron content. In all samples iron was determined by means of spectrophotometric method with sulfosalicylic acid. The method was realized with the aid of an original flow system allowing the analytical calibration to be performed with high reliability. As a result iron present in a sample in trace amount was able to be found with very good accuracy and with precision not exceeding 1.5% (in RSD). It was stated that typical amount of iron in a single cigarette is ranged between 0.3 and 0.4 mg. When smoking the risk should be taken into account that even 99% of this amount is transferred to human body without any chance to be limited by the cigarette filter.

**Key words** cigarette tobacco • iron • flow system • calibration

### Introduction

It is well known that the excessive amount of iron in the human body can contribute to many diseases including cancer. Certainly, one of the potential sources of toxic iron is the cigarette smoke.

Iron present in the human organism in ferrous state reacts with oxidizing reagents (e.g. with hydrogen dioxide) producing free radicals. Under normal conditions the radicals formed are controlled and removed by natural antioxidants but if the iron concentration systematically increases they are accumulated. Thus, iron overload may be a result of a high free radicals production, leading to hepatotoxicity via lipid peroxidation and the destruction of the hepatic mitochondria.

It was found that the lungs of cigarette smokers contain increased concentrations of iron in the form of extracellular ferritin-bound iron [6]. It can potentially be released by reductants present in cigarette smoke and catalyze generation of highly reactive hydroxyl radicals, which could then promote oxidative injury to lung cells.

In very recent reports, a comprehensive mechanism for the induction of oral cancer by cigarette smoke is suggested [1, 7]. Namely, synergistic effect of the cigarette smoke and saliva is postulated as being based on the reaction between redox active metals (including iron [1]) in saliva and low reactive free radicals in the cigarette smoke. As a result highly active hydroxyl free radicals are produced allowing saliva to lose its natural antioxidant capacity. Thus, saliva becomes a peculiar mixture being in fact more dangerous for human organism than the smoke alone.

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In the light of above facts, the analytical task to estimate carefully and reliably how much of iron could be transferred and absorbed as a result of cigarette smoking appears to be an important task. As trace amounts of iron have to be taken into account, an analytical method applied should be characterized by very good sensitivity. Additional evident requirement is to determine such a small amount of iron with so good accuracy and precision as possible. In order to achieve so good analytical conditions, it is necessary to perform analysis according to appropriate, optimized analytical procedure, including preparation of a sample and calibration.

The analytical procedure used in the present examinations encompassed the microwave digestion of the sample assayed and the determination of iron by means of spectrophotometric method with sulfosalicylic acid. The sample digested was chemically treated and introduced to the detector in the flow mode with the use of dedicated flow system designed in our laboratory. Using the same system the calibration was performed in such a way that a series of standard solutions were generated from a single standard and the sample diluted to different extent was exposed to measurements.

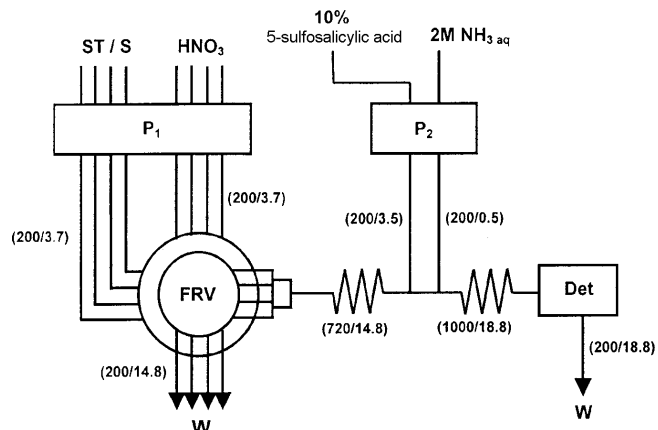
## Methods

Stock standard solution of 1000  $\mu\text{g/l}$  Fe was prepared from Titrisol standard. Working standard solution was prepared by dilution of the stock solution with 1%  $\text{HNO}_3$ . Solution of 5-sulfosalicylic acid, 10%, was made by water-dissolving of the adequate amount of  $\text{C}_7\text{H}_6\text{O}_6\text{S}\cdot 2\text{H}_2\text{O}$ . Solution of ammonia, 2 M, was prepared by water-dilution of concentrated  $\text{NH}_3$  aq. Solutions of nitric acid of required concentrations were made by water-dilution of concentrated  $\text{HNO}_3$ . All reagents used were of analytically pure quality and produced by Merck, Germany. Doubly distilled water was used throughout.

The following samples were taken for analysis: a) tobacco of cigarettes Marlboro, L&M, and Popular, b) tobacco ash of the same cigarettes, c) filters from cigarettes Marlboro and L&M, d) tobacco reference material "Oriental Tobacco Leaves" CTA-OTL-1 (Institute of Nuclear Chemistry and Technology, Warsaw, Poland). The tobacco samples were preliminarily dried at a temperature of 110° for 30 min, then ground in a laboratory mortar and dried again at 110° for 30 min. After such preparation they were stored in polyethylene bottles. All samples were digested with the use of a microwave oven UniClever BM-1z (Plazmatronika, Poland).

Analytical examinations were performed with the use of the flow system presented in Fig. 1. Solutions of the standard, sample and reagents were propelled with the use of two multichannel peristaltic pumps (LZ 1010, Zhaofa, China) with Tygon pump tubes. The fully rotary valve was a model LZ-1020 eight-positional and eight-channel injection valve (Zhaofa, China). PTFE tubings were used for all connections and reaction coils. A model SPEKOL 11 spectrophotometer (C. Zeiss, Germany) was used for detecting of iron at 425 nm.

Detailed information of how the system works and how the calibration procedure is carried out can be found elsewhere [2, 3].



**Fig. 1.** The flow manifold used for spectrophotometric determination of iron in the samples of cigarette tobacco: S – sample solution; ST – standard solution; W – waste; FRV – fully rotary valve; P<sub>1</sub>, P<sub>2</sub> – pumps; Det – UV/VIS spectrophotometer; in parentheses the values of tubing length (mm) and of flow rate (ml/min) are given.

## Results

The analytical method developed was preliminarily tested in terms accuracy and precision. For this aim the samples of the tobacco reference material were analyzed.

A portion of ca. 0.5 mg of a tobacco sample was introduced into a PTFE vessel and 1.5 and 4.5 ml of concentrated chloric and nitric acids, respectively, were added. The vessel was closed and after 1.5 h it was heated for 40 min with maximum pressure of 4500 kPa. The digested sample was slowly heated in a sand-baths until acids and oxides evaporated and after cooling it was transferred into a 100 ml volumetric flask and diluted to the mark with water.

For the calibration purpose, a single standard solution of 8 mg L<sup>-1</sup> Fe was prepared. It was introduced to the flow system through four tubes as seen in Fig. 1. When the FRV was operated (i.e. rotated gradually through 45° clockwise of four successive positions and back) two sets of five standard solutions of concentrations 8, 6, 4, 2, and 0 mg L<sup>-1</sup> Fe were generated one after the other, merged with reagents and delivered to the detector. The signals measured served for preparation of the calibration graph. Then, the sample solution was treated in the same way as the standard solution was. As a consequence, the sample undiluted and diluted 4/3, 2 and 4 times was successively prepared and exposed to measurements. On the basis of measurement data gathered four estimations, C<sub>1</sub>–C<sub>4</sub>, of the iron concentration in the sample were calculated and the averaged value was considered as the final analytical result. The calibration procedure described was applied as a whole for each sample individually.

In order to estimate the precision of the method used three samples were analyzed in different days. In Table 1 the results obtained are given in the form of particular estimations of the iron concentration in the sample and of the final concentrations. As seen, the latter ones are very similar to each other (3.57, 3.55, 3.57 mg L<sup>-1</sup>) and characterized by the relative standard deviation less than 1.5%. The amount of iron found in the reference material was

**Table 1.** Results of the precision examination.

Sample	Fe concentration found (mg L <sup>-1</sup> )					RSD (%)
	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	$\bar{C}$	
1	3.57	3.58	3.57	3.55	3.57	0.27
2	3.61	3.57	3.50	3.51	3.55	1.47
3	3.58	3.62	3.56	3.55	3.57	0.89

989.8 ppm. This value was different from the expected value (989.0 ppm) of only 0.13%.

Assuming that the analytical procedure has been optimized the essential examinations were carried out.

The samples of tobacco taken from cigarettes Marlboro, L&M, and Popular as well as the samples of tobacco ash were digested and prepared in the same way as the samples of the reference material. A sample of tobacco ash was taken for digestion from eight cigarettes of a given brand just after smoking of them. The filters were analyzed before and after smoking. A filter sample (taken from a single cigarette) was preliminarily treated with 4 ml of concentrated HNO<sub>3</sub> for 15 min and then digested for 15 min only (but not for 40 min) in the same conditions as the samples of tobacco and ash were. Further steps of the analytical procedure (realized with the use of the flow system) were the same as described previously.

The results obtained are presented in Table 2. As seen, a single cigarette among those being examined contained iron in amount between 0.3 and 0.4 mg. After smoking, iron was found in tobacco ash in amount ranged from 1 to 14% of the initial content. No significance differences in above results were noticed when “light” and “normal” kinds of Marlboro and L&M cigarettes were considered. The iron amount in filters was found to be much greater than in tobacco (especially in “normal” kinds) but it was kept practically at the same level before and after smoking of the cigarettes examined.

## Discussion and conclusions

The analytical method developed for the purpose of the present examinations allowed iron to be determined in tobacco and filters of selected brands of cigarettes with very good sensitivity, precision and accuracy. Certainly, one of the factors contributing to such successful results was the flow mode applied for chemical treatment of a sample

and for calibration of iron determination. It has been revealed many times that the flow technique is able to improve analytical performance of batch methods commonly applied in laboratory practice. This is mainly owing to the possibility of adjusting and controlling of the instrumental and chemical conditions at well defined optimized levels as well as of preparing of a sample with very good repeatability and reproducibility. The flow technique reveals also many abilities and advantages in the field of analytical calibration [4].

In general, the calibration seems to be underestimated stage of an analytical procedure. When realizing that the calibration is in fact the transformation of the measurement data to the analyte concentration, the reliability of the final analytical results depends on both the random fluctuations and systematic drifts of the signals measured. In the case of the calibration method applied here the latter effect was minimized due to the facts that each sample was measured four times and all signals were related to the calibration graph prepared individually. Furthermore, it was revealed that the standard solutions generated by the presented means lead to the calibration graphs which are self-corrected in terms of random errors caused by instrumental factors [3].

On the basis of the examination performed it can be stated that the amount of iron contained in a single cigarette is about 0.4 mg and the enormous majority of iron present in cigarette tobacco can be relocated to the human organism. This effect cannot be expected to be reduced either by using of cigarettes with filters or by smoking cigarettes of the “light” kind. If taking into account that a smoker is capable of smoking 20–25 cigarettes a day, the amount even up to 8–10 mg of iron can be absorbed during this time, while a normal amount is estimated at the level of 1 mg only [5]. Thus, it is clear that the cigarette smoking can be a serious source of excessive amount of iron in human body and consequently the reason of many diseases. The problem becomes evidently more serious when dealing with pregnant women and children.

The question worth to be examined is still how much of the iron amount transferred is able to be absorbed by human organs. However, even if most of iron introduced to the respiratory system is able to return with the smoke exhaled, it has a special chance to stay in the oral cavity and to be absorbed by saliva. In the light of last report [1, 7] just this phenomena is potentially very dangerous for human health.

**Table 2.** Results of the determination of iron in both tobacco and filters of the cigarettes examined.

Cigarette brand	Cigarette kind	Fe concentration found (mg L <sup>-1</sup> cigarette)			
		Tobacco	Tobacco ash	Fresh filter	Uses filter
Marlboro	normal	0.41	0.044	1.02	1.02
	light	0.43	0.042	0.76	0.61
L&M	normal	0.41	0.006	0.99	0.92
	light	0.40	0.004	0.48	0.45
Popular*	normal	0.33	0.047	–	–

\* Cigarettes are produced without filters.

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